

Remarks/Arguments

Claims 9, 15 and 16 are cancelled. New claims 25-32 are added. Therefore, claims 17-32 are pending. Claim 21 is amended to specify that the targeting vector includes homology arms directing the targeting vector to a specific chromosomal location. New claims 25-28 are drawn to an *in vitro* method of increasing targeting frequency in a mouse ES cell. Support for claim 25 is found in the specification at paragraph [0003]. Support for new claim 29 is found in the specification at paragraph [0004]. Support for claims 26-28 and 30-32 is found in original claims 4-8.

In the Advisory Action dated 24 May 2006, the Examiner entered the Amendment and Remarks document filed after Final Rejection and withdrew the rejections under 35 USC § 112, first paragraph. The claims remain rejected under 35 USC § 103(a), which rejection is addressed below. Applicants further herein submit a Declaration under 37 CFR § 132 by Dr. David Friendewey in support of the non-obviousness of the claims.

Rejections under 35 USC §103(a)

Claims 9 and 15-24 were rejected as being unpatentable over Rohozinski et al. (Genesis 2002 32:1-7), in view of Tsirigotis et al. (BioTechniques 2001 31:120-130) and Ghazizadeh et al. (1998) J. of Investigative Dermatology, 111:492-496. Claims 9, 15 and 16 are canceled. This rejection is respectfully traversed.

The invention as claimed

Claims 17-20 are drawn to an *in vitro* method of directing a targeting vector to a specific chromosomal location within a genome of a mouse embryonic stem (ES) cell, comprising introducing into the cell a targeting vector, wherein the targeting vector comprises a drug resistance gene under control of a ubiquitin promoter and homology arms directing the targeting vector to a specific chromosomal location. Claims 21-24 are drawn to the targeting vector used in the method of the invention. New claims 25-28 are drawn to an *in vitro* method of increasing targeting frequency in mouse embryonic stem (ES) cells, comprising introducing into a mouse ES cell a targeting vector, wherein the targeting vector comprises a drug resistance gene under control of a ubiquitin promoter, and homology arms directing the targeting vector to a specific chromosomal location. New claims 29-32 are drawn to an *in vitro* method of increasing the number of mouse embryonic stem (ES) cells correctly targeted with a targeting vector, comprising introducing into a mouse ES cell a targeting vector, wherein the targeting vector comprises a drug resistance gene under control of a ubiquitin promoter, and homology arms directing the targeting vector to a specific chromosomal location.

The Cited Prior Art

Rohozinski et al. Rohozinski et al. describe gene targeting via homologous recombination which targeted 2 genes on the Y chromosome. Tsirigotis et al. describes construct comprising a human ubiquitin C promoter and a GFP reporter gene. Ghazizadeh *et al.* describe using a retrovirus vector comprising the lacZ gene and the neomycin phosphotransferase gene to select non-transformed porcine keratinocytes using G418 to select cells which are not expressing neomycin phosphotransferase.

The analysis under §103(a)

A careful analysis of the prior art shows that none of the cited references disclose or suggest the use of a targeting vector comprising a ubiquitin promoter. Tsirigotis et al. discloses a construct using a ubiquitin promoter in a non-targeting vector used for random integration. Tsirigotis et al. do not disclose a targeting vector having a ubiquitin promoter. Rohozinski et al. describe a method of gene targeting in mouse ES cells via homologous recombination. Rohozinski et al. do not disclose a targeting vector having a ubiquitin promoter. Ghazizadeh et al. describe a retrovirus vector comprising a neomycin phosphatase gene. Ghazizadeh et al. do not disclose a targeting vector having a ubiquitin promoter.

In summary, although the individual components used in the instant method were known in the art, nothing in the art teaches or suggests making the specific combination of components, namely a targeting vector having a drug resistance gene under control of a ubiquitin promoter and homology arms directing the targeting vector to a specific chromosomal location. Importantly, the prior art did not discover that the use of such a targeting vector would provide the surprising and unexpected effects of improving correct targeting frequency in mouse ES cells, as well as allowing the targeting of genes that were not previously targeted by conventional targeting vectors.

In support of the nonobviousness of the instant claims, applicants submit a Declaration under 37 CFR § 1.132 by Dr. David Friendewey. The Declaration provides Table A which is an improved presentation of the data shown in original Table 2 and includes data not provided in the original table of results obtained with a third experimental vector having an SV40 promoter. The data generated with the SV40 promoter targeting vector was generated at the same time as the data presented for PGKp and hUBCp, but were not included in the original Table.

In the first column of Table A is provided the full names, symbols, and Entrez gene identification number of the targeted genes which are referred to in Table 2 as "N", "F", "P", etc. The second (PGKp), third (hUBCp) and fourth (SV40) columns provide the number of drug resistant ES cell clones. The fifth (PGKp) and sixth (hUBCp) columns provide the number of

clones determined to be correctly targeted by each targeting vector and the number of total clones screened (also expressed as a percentage). The last column provides a measure of the increased targeting frequency achieved with the ubiquitin promoter relative to the PGK promoter.

The PGK promoter is the promoter most frequently used by the art to drive expression of a selectable marker in production of transgenic mice. SV40 is a promoter commonly used in molecular biology. Prior to the instant discovery, the ubiquitin promoter was known (see Schorpp et al. (1996) *Nucleic Acids Res* 24:1787 cited at paragraph [0002] of the instant specification). However, until the experiments described in the instant specification were made, it was not realized that use of the ubiquitin promoter in a targeting vector for generating desired genetically modified mouse ES cells could increase targeting frequency and further, allowed genes to be successfully targeted which were not targeted by the PGKp vector.

In support of the surprising and unexpected results achieved by the instant invention, the Examiner's attention is directed to Table A of Dr. Friendewey's Declaration. A comparison of the targeting frequency (columns 5-7) show that the vector containing the ubiquitin promoter yielded a 6.5-fold increase for the F-box protein 25 gene; a 3.6-fold increase for the parathyroid hormone receptor 2 gene, a 2.4-fold increase for the cyclic AMP-regulated phosphoprotein gene; a 3.5-fold increase for interleukin 18 receptor accessory protein gene; a 2-fold increase for IL-20 gene; and a 4.75-fold increase for the Eph receptor A6 gene. These data were originally shown in column 7 of Table 2).

Further, as stated by Dr. Friendewey at paragraph 7 of the Declaration, and shown in the specification at lines 3-5 of paragraph [0042], and Table 2, column 5 for "N" and "S" genes (Table A, columns 5 and 6 for nebulin and interleukin 1 receptor-like 1 genes), use of the ubiquitin promoter in a targeting vector allows those genes to be targeted that were not targeted with the PGKp vector.

This discovery is of practical significance in that increasing the number of correctly targeted mouse ES cells dramatically reduces the amount of work needed to generate the desired genetic modification in a genetically modified mouse. Moreover, in some cases, it allows modifications to be made that had not been made previously.

Applicants further note that the effect seen with the ubiquitin promoter on improved targeting frequency does not appear to be the result of improved promoter strength. As stated by Dr. Friendewey in the Declaration, the increase in targeting frequency (6.5-fold) was not accompanied by an increase in expression of the neomycin resistance gene, as determined by mRNA quantification for the targeted F-box protein 25 locus.

Accordingly, in light of the above remarks and accompanying Declaration under 37 CFR

§ 1.132 from Dr. Friendewey, it is believed that this rejection should be withdrawn.


Conclusion

It is believed that this document is fully responsive to the Office action dated 2 March 2006. In light of the above amendments and remarks, it is believed that the claims are now in condition for allowance, and such action is respectfully urged.

Fees

This Amendment is filed with a Request for Continued Prosecution under 35 USC § 132(b), the fee for which is \$790. The Final rejection was dated 2 March 2006 and provided a 3 month response period to 2 June 2006; accordingly applicants petition for a two month extension for time to 2 August 2006, which is \$450. Accordingly, the Commissioner is herein authorized to charge Deposit Account Number 18-0650 in the amount of \$1240. In the event the Patent Office determines that additional fees are due, the Commissioner is hereby authorized to charge Deposit Account Number 18-0650 in the amount of any fees deemed to be due.

Respectfully submitted,


Valeta Gregg, Reg. No. 35,127
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
Direct Tel.: (914) 593-1077